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RESEARCH ARTICLE

PHYSIOLOGICAL AND SEED HEALTH TESTING OF RICE SEED (ORYZA SATIVA L.) GROWN IN THE DALOA AREA (CÔTE D'IVOIRE)

Tonessia Dolou Charlotte, Traore Brahima, N'goran Koffi Désiré, Soumahin Eric Francis, Dro Bernadin and Akaffou Doffou Selastique
Université Jean Lorougnon Guédé, UFR Agroforesterie, Laboratoire de l'Amélioration de la Production Agricole, Daloa, Côte d'Ivoire.

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Abstract

A survey was realized during the period of June, 2021 to August, 2022 in Daloa, Côte d'Ivoire to understand the rice cultivation practices. Then rice seed samples were collected to perform their health and quality, Dry inspection and blotter tests. Eight varieties of rice were collected, of which Wita 9, BkéAmlre and C15 are mainly grown under both rainfed and irrigated lowland. The water content of the rice seed samples collected fluctuated between 9 and 10 % showing that the farmers have a good command of post-harvest activities. the inert matter up to 56% and weed seeds in a mixture up to 9.47 % were found in rice seed samples collected. Although, these seeds showed good germination capacity with an average rate of 90%, among the seedlings having emerged, some presented color anomalies of the stem, collar and cotyledons ranging from 3 to 46%. The rotten grains (1.25 - 11%) invaded by molds and hard grains that remained intact (0.65 - 4%) throughout the test were found. Ten seed-borne fungi were detected from these seed samples. Some of the identified fungi could be associated with seedling abnormalities and grain rots during the germination.

Introduction:-

Rice (Oryza sativa L.) is the staple food for more than half of the world's population(Folefack, 2014). It ranks as the second most cultivated cereal in the world after wheat (Osanyinlusiand Adenegan, 2016). In Africa, rice has become a very strategic and priority product for food security. Its consumption is growing faster than any other commodity on the continent due to significant population growth, rapid urbanization and changing dietary habits (Seck et al., 2013). The key problem facing the rice sector in Africa is that in general local production has never matched demand. The continent therefore continues to depend on imports to meet the growing demand for rice (Harold and Tabo, 2015).

In Côte d'Ivoire, rice yield was 1,650 t in 2017 and only satisfied 10% of national demand (FAO, 2017). In other words, rice presents an issue of great importance for food security (Dembélé, 2001). Given the existing potential and the weight of imports, the country must reverse the trend by implementing a strategy for promoting rice cultivation. However, one of the major constraints in improving rice cultivation is the almost permanent deficit in agricultural inputs (Osanyinlusiand Adenegan,2016), more particularly in quality seeds (Sperling et al., 2004). Seeds are an

Corresponding Author:- N'goran Koffi Désiré
Address:- Université Jean Lorougnon Guédé, UFR Agroforesterie, Laboratoire de l'Amélioration de la Production Agricole, Daloa, Côte d'Ivoire.

essential element of any agricultural production. Rice varieties adapted to local cultivation conditions and seeds of good physiological quality and free from pathogenic germs are a guarantee for improving rice productivity and yield(Aminou and Aliou,2013).

Indeed, rice cultivation is hampered by many diseases among which dominate seedborne diseases(Dembélé, 2001; Sy and Séré, 1996). Among seed-hosted organisms, fungi cause maximum damage such as rotten, transformed, necrotic, discolored seeds with reduced germination and vigor. According to Sy andSéré (1996), seedborne diseases are considered to be the main causes of the reduction in the quality, quantity and consequently grain yield of rice. The use of quality seeds would increase any agricultural production by 10-15% (Akintayo et al., 2008) provided that the three main aspects of seed quality are met. These include genetic and physical purity, germination and seed vigor and health (Seshu and Dadlani, 1989).

Seed health mainly refers to the presence or absence of pathogenic organisms such as fungi, nematodes, bacteria, viruses and insects associated with seeds(ISTA, 1985). Farmers often use seed lots that are contaminated or mixed with impurities for their cultivation, thus compromising their agricultural yield(Fujisakaet al., 1993). Seed borne pathogens are one of the main causes of low cereal productivity. Improving the quality of seeds, and particularly that of their health condition, is one of the factors that can contribute to creating the conditions for the sustainable development of the rice sector in Côte d'Ivoire. In this study we are interested in the locality of Daloa in ‘‘Sassandra’’, a region known to be one of the rice granaries of Côte d'Ivoire. We formulate the hypothesis that the seeds of rice varieties cultivated in the locality of Daloa are responsible for the low rice yield. This study is a contribution to the improvement of rice productivity through a better knowledge of the rice-growing environment and the health quality of seeds.

Materials and Methods:-

Study area and plant material

This study was carried out in the locality of Daloalocated between longitudes 6°27’ West and latitudes 6°53’ North. The climate of this locality is of the humid and hot tropical type (Anonymous, 2016). The temperature varies from 24.65°C to 27.75°C on average, with small annual amplitudes of around 5°C. The average rainfall over the past ten years is 1302.23 mm of rain per year. These favors sustained plant growth throughout the year. Overall, the average humidity in this locality is around 86.01% with a minimum of 56% in December and January**.**The plant material used consisted of rice seed. These seeds came from rice varieties cultivated by farmers on 4 sites in the rice-growing areas. These included Bata (West of Daloa part), Gonaté (East of Daloa), Gako extension (South of Daloa) and Zepréguhé (North of Daloa). These sites are divisions of areas that may include a number of rice farms ranging from 50 to 250 in which producers are grouped together. In the first time a survey was carried out in the rice-growing perimeters of the localities of Daloa. The second method consisted in testing in the laboratory the physiological and health quality of the seeds collected.

Survey of farmers’ practices

The survey was carried out in 2021 and 2022. It consisted in questioning rice farmers directly from a questionnaire sheet previously established. The main information sought related to the names of the varieties of rice cultivated, the type of rice cultivation practiced, the size of the plots and the estimate of their yield. Then seeds sampled were kept in envelopes which bore the information relating to the date of collection, the name of the variety, the site, the name and the contact of the farmers. The different samples were brought to the laboratory to their analyze.

Physical quality of seeds

This involved studying the specific purity of the seed samples. Thus, 500 g from each sample were manually inspected and graded into three categories. The categories were pure seeds (specific purity), mixed seeds of other plants and inert matter.The different categories were expressed in percentage according to the following formula:

$$\text{Purity } (\%) = \frac{\text{Average mass of a sorted element}}{500} \text{X}100$$

Seed water content

100g samples of pure seed of each variety were weighed and replicated ten times (M1).The samples were put in aluminum foil and placed in an oven with sufficient spacing to allow good air circulation. The oven was then maintained at a temperature of 130° C for one hour. Finally, the seeds were cooled and weighed again (M2). The water content (WC) was determined according to the following formula:

$$WC\ (\%) = \frac{M1 - M2}{M1} X100$$

Germination test

Four hundred (400) grains of rice per sample were sown in Petri dishes at the rate of 25 seeds perPetri dish. Each sample was dampen and paper and seeds were absorbed water for about an hour. Additional water was added to dish just prior to placing into growth chamber. The seeds, which had not undergone any preliminary treatment, were spread on blotting paper and moistened with sterile distilled water after deposit of the seeds. Each variety was repeated 4 times. Observations began from the 3rdday after sowing until the 7thday and focused on:

- ✓ Normal seedlings (vigorous seedlings with no abnormalities)
- ✓ Abnormal seedlings
- ✓ Hard seeds
- ✓ Rotten seeds.

The rate of each group (P) of seedlings obtained was calculated as follows:

$$P\ (\%) = \frac{NSN}{TNSS} X100$$

NPN = Number of seedlings obtained in each group, TNSS = Total number of seeds sown

Incubated seeds on blotting paper (disc-shaped)

The blotting paper method was applied (ISTA, 2011). It consisted in testing 400 grains of pure seed at the rate of 25 grains per 90-mm diameter Petri dish. The grains were spread over two rounds of filter paper placed on top of each other and moistened with sterile distilled water after deposit. The seeds were placed in three circles, at the rate of 15 on the periphery, then 9 in the middle and one in the center. After 7 days of incubation at 28°C under the appropriate conditions, the grains were examined using a stereoscopic microscope to observe the presence of microscopic fungi.

Identification of seedborn fungi on agar medium

The fungi whose spores did not germinate well on blotting paper were cultured on Potato dextrose agar (PDA) medium for their identification. In this study, we investigated light conditions and suitable medium conditions using the slide culture method to establish optimal conditions for continuous spore acquisition of fungi according to Makunet al. (2007).

Statistical analysis of data

All data were entered with Excel 2010. The one-way ANOVA test was used to reveal the significant differences between the varieties depending on the sites. The classification of the means was carried out by the Tukey HSD test at 5% threshold. Statistical analysis of the collected data was done using Statistica 7.1 software. The significance level was set at $p< 0.05$. Seed-hosted fungi were identified based on their conidiospore structure and the identification key used was Common Laboratory Seed Health Testing Methods for detecting Fungi (Mathur and Kongsdal, 2004).

Results:-

Characteristics of rice varieties cultivated

The study made it possible to interview 122 rice farmers in the department of Daloa. These rice growers cultivate 8 varieties of rice spread over 2 types of rice cultivation which are rainfed lowland rice and irrigated lowland rice (Table 1). The varieties of rice found are wita9, V10, Bkéamlre, C35, C15, Bouaké189 (Bké 189) and two traditional varieties called ‘‘riz 3 mois and riz 5 mois’’. These eight varieties can be classified into early varieties, medium cycle varieties and late varieties. The most cultivated variety in the Daloa area was Wita 9, then BkéAmlre and C15. These varieties are those that would give a high yield between 1 and 4 T/ha. Wita 9 was cultivated under the 2 types of rice cultivation with an estimated surface area of 69.25 ha. The least cultivated varieties were C35 and Riz 5 mois.

Table 1:- Characteristics of rice varieties cultivated in the Daloa area.

Class	Cultivatedvarieties	Crop cycle(Days)	(%)User farmers	Estimated surface area (ha)	Estimatedyield (T/ha)	Type of rice cultivation
Early cyclevarieties	Riz 3mois	90	1.64	3	1	Rainfedlowland
	Wita 9	110	69.67	69.25	4.03	Irrigatedlowland, Rainfedlowland
Medium-cycle varieties	C15	120	10.66	4.00	1.02	Irrigatedlowland
	BkéAmlre	125	12.30	45.00	3.46	Irrigatedlowland
	Bké 189	130	0.82	5.00	1	Irrigatedlowland
	C35	130	1.64	1.00	0.50	Rainfedlowland
	V10	135	0.82	4.00	1.00	Rainfedlowland
Long-cycle varieties	Riz 5mois	150	2.46	1.5	0.5	Rainfedlowland

Nbr =Number

Specific purity of samples of rice varieties cultivated in the locality of Daloa

The sorting of the rice samples made it possible to observe three parts which are the pure seeds, the inert matter composed of plant debris and stones and finally the seeds of weeds mixed in the samples (Figure1). The rate of these three partsvariesdepending on the harvesting sites (Table 2).

Figure1:- Different parts obtained after sorting in the seed lots.
A: specific purities B: Inert material C: Weed seeds

Table 2:- Specific purities of the different varieties sampled.

SITES	Cultivatedvarieties	(%) SP	(%) MI	(%) WS
BATA	WITA9	77,64a	4,2c	9,47a
	BkéAmlré	92,68a	6,28c	0b
	Bké 189	91,47a	8,48c	1,09a
	5mois	91,04a	8,58c	0b
	C15	87,12a	16,24b	0,65b
GAKOEXTENSION	BkéAmlré	67,68b	30,32a	0b
	Bké 189	80,50a	21,27a	0b
	WITA9	84,72a	15,26b	0b
ZEPREGUHE	C35	80,92a	19,3b	0,1b
	V10	91,48a	6,96c	1a
	WITA9	77,28a	22,66a	0,06b
GONATE	3mois	88,02a	11,98b	0b
	5mois	90,61a	8,09c	0b
	Bkéamlré	46,44b	53,56a	0b
	WITA9	80,56a	18,41b	0b

PS = Pure Seed, WS= Weed Seed; MI = Inert Matter. In each column for the same site, the values followed by the same letter are not significantly different at $p < 0.05$ (tukey's HSD test)

Moisture content of rice seeds of the different varieties
Table 3 gives the average water content of the seeds of the varieties. The values fluctuated between 9% and 10% for all varieties and regardless of the site.

Table 3:- Water content of the 8 varieties of rice.

SITES	Cultivatedvarieties	(%) Water content
Bata	Wita 9	10
	Bkéamlré	10
	Bké 189	9
	5mois	10
Gonaté	Wita 9	10
	5mois	10
	3mois	10
Gako-extension	C15	10
	Bkéamlré	9
	Bké189	10
	Wita 9	9
Zépréghué	Wita 9	10
	V10	10
	C35	10

Germination quality of the seeds
The germination test lasted 7 days and made it possible to distinguish a good germination capacity of the rice seeds with an average rate of 90%. However, abnormalities were observed (Figure 2). Among the seedlings having emerged, seedlings with color abnormalities of the stem, collar and cotyledons were observed (Figure 2A) and varied depending on the study sites from 3 to 46%. Rotten grains (1.25 and 11%) invaded by molds and hard grains that remained intact (0.65 - 4%) throughout the test were also observed. Abnormalities were particularly high in BkéAmlre (Table 4).

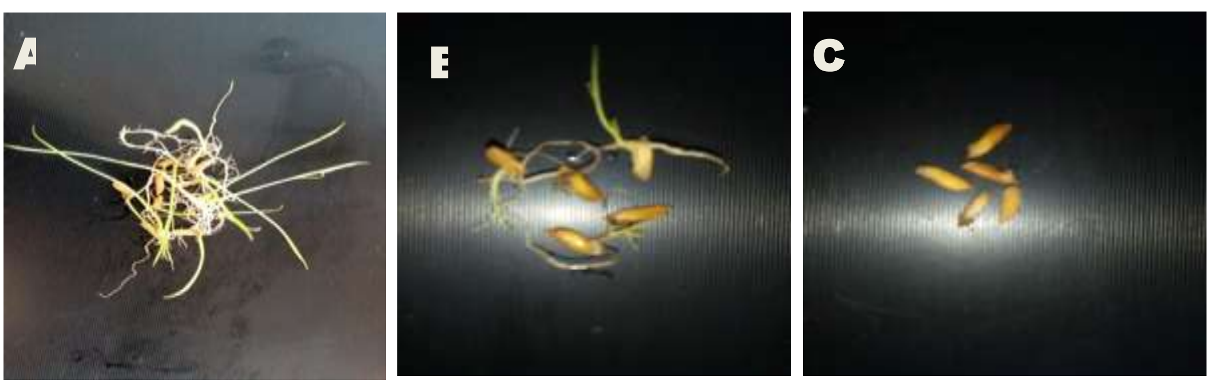

Figure2:- Different germination abnormalities.
Normal Seedlings (A), Abnormal Seedlings (B), Rotten grains (C)

SITES	CultivatedVariéties	NS	AS	RG	HG
BATA	Wita9	78a	6c	4b	2b
	Bkéamlré	28b	46a	11a	15a
	5Mois	85a	9b	5b	1b
	Bké 189	74a	15b	10,33a	0,67b
GAKO EXTENSION	C15	94a	3b	2b	1b

	Bkéamlré	75a	15a	6a	4a
	Wita9	93a	4,5b	2b	0,5b
	3mois	75a	14a	7a	4a
	5mois	85a	5b	6a	4a
GONATE	Wita9	76a	16a	4a	4a
	C35	87a	7a	4a	2a
ZEPREGUHE	Wita9	94a	4,25b	1,25b	0,65b
	V10	94a	4,25b	1,25b	0,65b

Table 4:- Germination quality (%) of seeds of the different rice varieties
NS= Normal seedlings; AS= Abnormal seedlings; RG= Rotten grains; HG= Hard Grains. In each column for the same site, the values followed by the same letter are not significantly different at $p < 0.05$ (tukey's HSD test).

Seed health test
The identification of fungi from rice grains under the microscope and on PDA medium made it possible to highlight 10 fungal species in the Daloa area. These included Fusarium monoliforme, Aspergillus Niger and Aspergilusgraminearums, Curvularialunata, Phomasp, Epicoccom nigrum, Mucor sp, BipolarisOryzea, Helminthosporium Oryzae and Alternaria alternata. Only the species Fusarium monliforme, Aspergilusgraminaerumand Alternaria alternata were observed on all varieties of the 4 sites out of these 10 species of fungi. The genus Phoma was observed at 1% on the seeds of the varieties 5 mois and Wita 9 from Bata. As for the Epiccorium species, it was identified on all the seeds of the varieties from Bata and Gako extension then on the seeds of the variety 3 mois from Gonaté. The species Helminthosporiumoryza was observed on the variety 3 mois harvested in Gonaté with an incidence of 31%.

Discussion:-

The survey carried out in the locality of Daloa revealed eight (8) varieties of cultivated rice. The farmers grow them and keep some of the seeds of the rice varieties for the following crop year. The analysis of these seeds showed that their physical quality was acceptable according to the standards defined by ISTA (2011). Indeed, the water content of the seeds collected was less than or equal to 10%. These results show that farmers have a good command of post-harvest activities and can preserve seeds for the long term. However, the seeds preserved by farmers contain many impurities, namely plant debris, stones and weed seeds. These mixed elements can depreciate the market value of the rice produced in the locality of Daloa and, in turn, compromise the yield (Fujisakaet al., 1993). Farmers therefore have an interest in cleaning their field in order to reduce weed seeds and protect rice samples from inert debris resulting from harvesting and rice drying. The mixed elements can also be reservoirs of harmful organisms likely to hinder the germination quality of rice seeds. This is probably the reason why the seeds studied, although having germinated at 90%, showed a non-negligible rate of seedlings with deformations or brown discoloration of the collar and/or stem as well as rotten seeds. These abnormalities are characteristic of certain fungi hosted by seeds and are responsible for dieback and lack of vigor of seedlings in the field (Angladette, 1966; Van-wyk et al., 1988).

The analysis of the sanitary quality of the rice samples revealed the presence of 10 fungal species on the seeds of the varieties studied. These fungal agents were either saprophytic fungi or parasitic fungi that infect seeds in the field before harvest. The group of saprophytes included Aspergilusniger, Aspergilusgerminearum, Mucor sp. Epicoccumsp and Nigrospora sp. These can cause sterility or even rotting of seeds and are generally linked to poor seed preservation conditions. The second group concerned Alternaria alternata, Fusarium moniliforme, Curvularialunata, Bipolaris oryzae, Helminthosporium. Their development requires the presence of sufficient temperature in the seeds (Makun et al., 2007). They are responsible for the accentuation of the discoloration of rice grains and an alteration of their quality (Durais-wamy and Mariappan, 1983). Thus, the germination of seeds contaminated by these parasites is affected and there follows a reduction in the vigor of the plants or their dieback (Bautistaand Opina, 1991).

Parasitic fungi can also attack aerial parts. Work has shown that these fungi are capable of causing typical leaf symptoms on different varieties of rice (Hannin, 2003; Ouazzani et al., 2000). All of them produce comparable alterations and can infect not only leaf organs, but also the floral parts (glumes, lemmas) hence the contamination of the grains. The resulting deterioration of seeds increases when the conditions of humidity and temperature are favorable to the development of such microorganisms (Bothast, 1978). The use of seeds heavily infected with the fungal agents that we have isolated can reduce the germinating power and the potential yield of rice plants and thus represent important factors of disruption of the sustainability of rice production systems.

Conclusion:-

The seeds of the varieties of rice mainly cultivated in the locality of Daloavarieties showed good specific purity and a low water content conducive to long-term seed preservation. However, the level of impurities mixed in the rice samples depreciates their quality. The germination rate of the varieties is high but the resulting rate of abnormal seedlings could be responsible for the low rice productivity and yield in the locality. The health analysis of the varieties made it possible to identify 10 fungal species. Some of these fungi are responsible for the abnormalities observed on rotten seedlings and seeds. Others like HelminhtosporiumOryseaandBipolarisOrysae can be responsible for major outbreaks in rice cultivation. In order to improve rice yield in the locality of Daloa, it would be important to treat rice seeds before any sowing, to clean the rice fields so as to reduce weed seeds in samples and monitor harvest and post-harvest operations to reduce inert debris and stones in samples.

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